Growth, flesh adiposity and fatty acid composition of Atlantic salmon (*Salmo salar*) families with contrasting flesh adiposity: effects of replacement of dietary fish oil with vegetable oils

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ABSTRACT

The present study compared the effects of diets formulated with reduced fishmeal (FM) content and either 100% fish oil (FO) or 100% of a vegetable oil (VO) blend in post-smolts of three family groups of Atlantic salmon. Two groups were selected as being either “Lean” or “Fat” based on estimated breeding values (EBV) for flesh adiposity of their parents derived from a breeding programme, while the third group (CAL) was a mix of non-pedigreed commercial families unrelated to the two groups above. The VO blend comprised rapeseed, palm and a new product, *Camelina* oil in a ratio of 5/3/2, and diets were fed to duplicate pens of each salmon group. After an ongrowing period of 55 weeks, to reach a mean weight of 3kg, the fish from all treatments were switched to a decontaminated FO for a further 24 weeks to follow restoration of long-chain n-3 polyunsaturated fatty acids (LC-PUFA) in the fish previously fed VO. Final weights were significantly affected by family group and there was also an interaction between diet and group with Fat and Lean FO fish being larger than the same fish fed VO. Specific growth rate (SGR) was highest in CAL fish (1.01), feed conversion ratio (FCR) was highest in the Lean fish but there were no significant effects on thermal growth coefficient (TGC). Condition Factor (CF) was lowest in CAL fish while the hepato-somatic index (HSI) was highest in Lean fish and viscero-somatic index (VSI) highest in Fat fish. Flesh and viscera lipid content was affected by both family group and diet with a significant interaction between the two. Flesh lipid in fish fed FO was in the order Fat > CAL > Lean although this order was Fat = Lean > CAL when fed VO. Flesh fatty acid compositions were affected mainly by diet although some minor fatty acids were also

influenced by group. Fish fed VO had n-3 LC-PUFA reduced by ~65% compared to fish fed FO but this could be restored by a 16 week FO finishing diet phase. The differences observed in lipid and fatty acid deposition suggested that genetics affected lipid deposition and metabolism and that breeding programmes could select for fish that retained more n-3 LC-PUFA in their flesh, particularly when fed diets low in these fatty acids.

Keywords: Rapeseed oil, palm oil, *Camelina* oil, genetic strain or family, fish oil, growth, fatty acid compositions.

1. Introduction

Worldwide demand for seafood continues to expand while traditional fisheries are at best stable or in decline, and recent evidence suggests that aquaculture production now provides half of all seafood (FAO, 2009). In recent times global production of Atlantic salmon increased from ~0.9 million metric tonnes (MT) in 2000 to 1.5m MT in 2006 and as such is the largest production of a marine fish species globally (FAO, 2008). As with most carnivorous fish, salmon has traditionally been cultured using diets rich in fishmeal (FM) and fish oil (FO), which has placed excessive demand on these raw materials, resulting in increased prices and concerns about ethical sustainability. Production of FO globally has ranged from 1.0m to 1.5m metric tonnes between 1980 and 2006 and there is no likelihood of this increasing (Tacon and Metian, 2008). Recent estimates of FO use by aquaculture suggested 87% of global production is currently utilised with salmonid culture being the major user at 66.4% of total FO production (Tacon et al., 2006). Tacon et al., 2006 suggested that aquaculture would use 88% of FO production up to 2012 while an earlier study suggested that 98% could be consumed by 2010 (Pike & Barlow, 2003). All of these predictions could be eclipsed if a severe El Nino event occurs in the next few years, as has been widely predicted. In summary, the need to establish alternatives to FM and FO has never been more urgent if aquaculture production is to increase and seafood supply and nutritional quality is to be maintained.

Several studies conducted recently have shown that terrestrial vegetable oils (VO), either singly or as blends, can replace some or all of the added FO in salmonid feeds largely without any compromise in growth or fish health and condition (Bell et

al., 2001, 2004; Torstensen et al., 2000, 2004a, 2005; Richard et al., 2006; Karalazos et al., 2007). However, replacement of FO, rich in the n-3 LC-PUFA, eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic acids (22:6n-3: DHA), with VOs devoid of n-3 LC-PUFA but rich in α-linolenic acid (18:3n-3) and especially linoleic acid (18:2n-6), can significantly reduce the flesh n-3 LC-PUFA concentration by more than 50% if total FO replacement is implemented (Bell et al., 2004; Torstensen et al., 2005; Richard et al., 2006). While salmon grown on diets rich in VO have reduced n-3 LC-PUFA, such products are still rich in n-3 LC-PUFA compared to most other fish species (Bell and Tocher, 2009). However, given the well established efficacy of n-3 LC-PUFA in preventing and attenuating a range of inflammatory disorders, including cardiovascular disease, immune dysfunction and neurological conditions, it is vitally important to maintain flesh n-3 LC-PUFA in salmon at levels close to those found in salmon fed largely on FO (Young and Conquer, 2005; Ruxton et al., 2005; Bell, 2008). Finishing diets rich in marine FO have been used to restore n-3 LC-PUFA levels in market size salmon that had previously been fed high levels of VO. Feeding FO finishing diets to fish previously fed VOs, for periods of 16-24 weeks, have successfully restored flesh levels n-3 LC-PUFA to 80-100% of their concentrations in FO-fed fish (Bell et al., 2004; Torstensen et al., 2005).

As described above, there is an extensive literature on the replacement of FO with both single and blended VOs in a range of fish species and there is a similar body of literature on the replacement of FM with plant proteins. In salmonids, partial or total replacement of FM with plant proteins has been demonstrated by a number of authors, although in some cases growth reduction was observed, which may have been due to

117 the presence of anti-nutritional factors that impacted on feed intake and gut function
118 (Kaushik et al., 1995; de Francesco et al., 2004; Espe et al., 2006). Interestingly, lipid
119 retention was increased in rainbow trout but decreased in Atlantic salmon when fed
120 high levels of plant protein (Kaushik et al., 2004; Espe et al., 2006). While the current
121 project focused more on the complete replacement of FO with VO on different salmon
122 family groups, the levels of FM and plant protein used were lower and higher,
123 respectively, than in commercial salmon diets used in 2006. Hence, a secondary aim
124 was to develop and test feed formulations that significantly reduced dependence on
125 marine resources and result in more sustainable aquafeed products.
126 The proximate composition of fish flesh, and especially the lipid content, has an
127 impact on the sensory quality of the flesh that also impacts on consumer preferences
128 that may vary between different geographic regions and cultures. It is therefore
129 important for the aquaculture industry to produce fish that use the dietary nutrients to
130 promote muscle growth over adipose growth as well as meeting consumer perceptions
131 in fulfilling optimal quality parameters. Thus, the ability to develop breeding
132 programmes in farmed fish that exploit desirable phenotypic and genotypic traits that
133 influence lipid deposition or adiposity has resulted in recent research activity (Neira et
134 al., 2004; Quillet et al., 2005; Tobin et al., 2007). There is also evidence that lipostatic
135 regulation can be influenced by dietary lipid and protein content in fat and lean strains
136 of fish and chickens (Bourneuf et al., 2006; Tobin et al., 2007; Kause et al., 2009).
137 Evidence suggests that retention of n-3 LC-PUFA subject to genetic control in poultry
138 (Mennicken et al., 2005).

The primary aim of this study was to grow Atlantic salmon smolts, for the whole marine production phase, on diets with reduced FM diets and that contained either 100% of added oil as FO or a blend of VOs. Each of these two diets was fed to either "Lean" or "Fat" salmon families as well as a commercial strain used in previous studies on FO replacement (Bell et al., 2004; Torstensen et al., 2005). Flesh fatty acid compositions were determined at final sampling for the ongrowing phase. Thereafter, all fish were placed on a FO diet, containing a decontaminated FO for the finishing diet phase, and flesh fatty acid concentrations measured after 8, 16 and 24 weeks on the FO finishing diet.

2. Materials and Methods

2.1 Fish Populations

Three genetically characterised and contrasting groups of farmed Atlantic salmon post-smolts were provided for the feed trials. Two groups comprised full-sib families selected from the Landcatch Natural Selection Ltd breeding program (Argyll, Scotland) based on estimated breeding values (EBVs) of their parents for high or low flesh adiposity. The parental EBVs were derived from Torry Fatmeter (Distell Industries, West Lothian, UK) lipid assessments (recorded from microwave reflectance of fat as a percent of body weight) obtained on live and harvested sibs of those parents. These assessments have a heritability (proportion of observed variation due to family differences) ranging from 0.17 to 0.39 in this dataset. Four unrelated full-sib families were then created from the selected parents at the November 2004 stripping season, two families from the extreme lower end of the EBV distribution for

Torry fat meter assessed lipid content ("Lean") and two families from the extreme upper end of the distribution ("Fat"). The average EBV for the lipid content as determined by Torry fat meter of the two Fat families was 2.00 percentage units higher than that of the two selected Lean families, representing a standardised selection differential of 2.33 standard deviations (sds). The corresponding standardised selection differential of the two Fat families for weight EBV at harvest was 0.62 sds ie the Lean families being genetically slightly heavier than the Fat families. A third group was of non-pedigreed mixed families derived from the Caledonian 20 strain (CAL; Marine Harvest Scotland Ltd.), which had been used in previous replacement trials (Bell et al., 2004; Torstensen et al., 2005).

Two thousand smolts of each of the three groups were stocked into 12 x $5m^3$ net pens at the Ardnish Fish Trials Unit (Marine Harvest Scotland, Lochailort, PH38 4LZ, Highland; 500 fish/pen) and the initial mean weights were 52, 88 and 85g for the CAL, Fat and Lean fish, respectively. Thus, there were 4 pens for each of the 3 groups with each of the two diets being feed to duplicate pens for each group. The temperature over the experimental period (May 2006 to December 2007) ranged from 5.5 to 17.0^{o}C with a mean temperature of 11.5 ± 2.9^{o}C.

2.2 Experimental diets

The fish were fed one of two experimental diets, prepared by Skretting ARC (Stavanger, Norway) in 3 different pellet sizes (3, 6 and 9 mm) for a period of 55 weeks until the fish reached a weight of ~3 kg. The diets were formulated to fully satisfy the nutritional requirements of salmonid fish (NRC, 1993). Duplicate pens of each of the 3 genetic groups were fed a similar basal diet containing either 100%

185 Northern FO or a VO blend comprising rapeseed, palm and *Camelina* oils in a ratio of
186 5:3:2. The oil of the plant *Camelina sativa* is a new addition to aquaculture feeds
187 although it has been used since Neolithic times as a lamp oil and also in cosmetics. It
188 is low in saturated fatty acids and contains 18:3n-3 and 18:2n-6 PUFA in a ratio of
189 ~2:1. It is unusual in being rich in the monoene fatty acid, 20:1n-9, representing
190 around 15% of the fatty acids and is more usually found in marine FO where it is a
191 preferred source of dietary energy. Its seeds contain more than 40% oil and it can be
192 grown in arid conditions not tolerated by other oilseeds and requires minimal use of
193 fertilisers, herbicides or pesticides (INFORM, 2007). The diets contained 25-32% FM
194 and 40-45% plant meals. After sampling at 55 weeks, 60 fish/pen for each group,
195 weighing between 2.0 and 3.5 kg, were placed in a single pen and the fish that had
196 previously been fed the VO diet were marked by adipose fin clipping. Thus, the
197 original 12 pens were reduced to 3 and these pens were fed a finishing diet, having the
198 same basal formulation as the 9 mm ongrowing diet but coated with a decontaminated
199 FO (sand eel/sprat oil, 9:1v/v; Triple Nine Protein, Denmark), for a further 24 weeks.
200 The diet formulations and proximate compositions of the 6 and 9 mm ongrowing feeds
201 are shown in Table 1. Fatty acid compositions of the 9 mm FO and VO diets and the 9
202 mm finishing diet are shown in Table 2. Diets were supplied by automatic feeders
203 controlled by an automated feed sensor system (ArvoTec, Sterner AquaTech UK,
204 Scotland). Feed use and mortalities were recorded and removed daily.

205 *2.2 Sampling procedures*

206 At 55 weeks, 25 fish were sampled per pen, killed by a blow to the head following
207 anaesthesia using MS222, and length, weight, viscera and liver weight recorded. From

ten fish/pen steaks from the Norwegian Quality Cut region (NQCs; a steak cut between the end of the dorsal fin to the vent) were collected and immediately frozen on dry ice and transported to the laboratory where they were stored for a short time at -20^{o}C before being processed. The steaks were thawed, skinned and deboned and then 2 flesh pools per pen, each comprising 5 fish/pool, were prepared for flesh fatty acid and proximate analyses. Samples of viscera and liver were prepared similarly. At the finishing diet sampling points, 24 fish per pen, 12 each from fish previously fed the FO or VO diets, were sampled after 16 and 24 weeks on the finishing diet. Four half NQCs were pooled to provide 3 replicates for each dietary treatment and genetic group. Samples were stored on dry ice for transport and stored and processed as described above.

2.3 Proximate compositions of diets and tissues

Moisture was determined by thermal drying to constant weight at 110^{o}C for 24h. Crude protein content was determined by Kjeldahl analysis (nitrogen x 6.25; Kjeltec Autoanalyser, Tecator, Hoganas, Sweden). Crude fat was determined in diets following acid hydrolysis using a Soxtec System 1047 hydrolysing unit (Tecator Application note 92/87) followed by exhaustive Soxhlet extraction using petroleum ether (40-60^{o}C boiling point) on a Soxtec System HT6 (Tecator application note 67/83). The crude fat in flesh, viscera and liver homogenates was determined by the above procedure but without acid hydrolysis. The ash content was determined by dry ashing in a porcelain crucible in a muffle furnace at 600^{o}C overnight. All methods are

based on those described in the Association of Official Analytical Chemists (AOAC) (2000) and modified as described in Bell et al. (2001).

2.4 Lipid extraction and fatty acid analysis

Total lipids were extracted from 1-2 g of pooled muscle or liver by homogenising in 20 volumes of chloroform/methanol (2:1 v/v) in an Ultra Turrax tissue disrupter (Fisher Scientific, Loughborough, UK). Total lipids were prepared according to the method of Folch et al. (1957) and non-lipid impurities were removed by washing with 0.88% (w/v) KCl. The weight of lipids was determined gravimetrically after evaporation of the solvent and overnight desiccation under vacuum. Fatty acid methyl esters (FAME) were prepared by acid-catalysed transesterification of total lipids according to the method of Christie (2003). Extraction and purification of FAME was performed as described by Ghioni et al. (1996). FAME were separated and quantified by gas-liquid chromatography using a Thermo Fisher Trace GC 2000 (Thermo Fisher, Hemel Hempstead, UK) equipped with a fused silica capillary column (ZB wax, 30 m x 0.32 mm i.d.; Phenomenex, Macclesfield, UK) with hydrogen as carrier gas and using on-column injection. The temperature gradient was from 50 to 150^{o}C at 40^{o}C/min and then to 195^{o}C at 1.5^{o}C/min and finally to 220^{o}C at 2^{o}C/min. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980). Data were collected and processed using the Chromcard for Windows (version 2.00) computer package (Thermoquest Italia S.p.A., Milan, Italy).

2.5 Calculations

253 The following formulae were applied to the data:

254 Specific growth rate (SGR) was calculated as % daily growth increase:

255 $SGR = (\ln W_1 - \ln W_0) \times (\text{days of trial}^{-1}) \times 100$

256 Where W_0 and W_1 are the initial and final body weights, respectively.

257 Feed conversion ratio (FCR) was calculated as the amount of dry diet consumed
258 and the total biomass gain:

259 $FCR = (\text{feed intake, kg}) \times (\text{wet weight gain, kg})^{-1}$

260 $\text{Thermal Growth Coefficient (TGC)} = [(W_1)^{1/3} - (W_0)^{1/3}] \times (\text{days} \times C^o)^{-1} \times 1000$

261 $\text{Condition factor (CF)} = W\ (g) \times (\text{fork length, cm})^{-3} \times 100$

262 $\text{Hepato-somatic index, HSI\%} = 100 \times (\text{liver weight, g}) \times W\ (g)^{-1}$

263 $\text{Viscero-somatic index, VSI\%} = 100 \times (\text{viscera weight, g}) \times W\ (g)^{-1}$

264 *2.6 Statistical analysis*

265 The significance of differences ($P < 0.05$) were determined by one-way (Figure 1
266 data) or two-way ANOVA (all other data sets). The latter was used to determine the
267 effects of diet, fish group and their interactions on growth parameters and lipid
268 compositions. In data analysed by one-way ANOVA differences between means were
269 determined by Tukey's test. Data identified as non-homogeneous (Bartlett's test) were
270 subjected to log or arc sin transformation before applying ANOVA. Statistical
271 analyses were performed using a Graphpad Prism™ (version 4.0) statistical package
272 (Graphpad Software, San Diego, CA).

273

274 **3. Results**

At the end of the 55-week feeding trial, there were no differences observed for final length or TGC but there was a significant effect of fish group on both SGR and FCR (Table 3). SGRs ranged from 0.89 and 1.01 with the highest values in CAL fish and lower values in the Lean and Fat fish. FCRs ranged from 1.06 to 1.39 with lowest values in CAL fish and the highest values in the Lean VO fish. SGR, TGC and FCR values are within normal ranges for cage-cultured salmon. There was a significant effect of group, and interaction between group and diet on the final fish weights, with lowest weights in the CAL FO and VO fish and the Fat VO fish, while the largest fish were the Lean and Fat FO fish. However, the lower final weight in the CAL fish may be due to them being only 52g at the start of the trial compared to the Lean and Fat groups which were ~87g (Table 3). The values for CF, HSI, and VSI were influenced by fish group. CF was highest in Fat fish and lowest in CAL fish while HSI was highest in Lean fish compared with Fat and CAL fish and VSI was highest in Fat fish compared to the Lean and CAL fish (Table 3).

The flesh lipid content was significantly affected by fish group and there was also a significant interaction between group and diet (Table 4). The Lean and Fat families were selected on the basis of their estimated genetic potential for muscle lipid content and the data from the fish fed FO supports this position with the order of lipid content being Fat > CAL > Lean (Table 4). However, when the fish were fed 100% VO this order was changed to Fat = Lean > CAL. The pattern of visceral lipid content was also significantly affected by group and there was a significant interaction between group and diet (Table 4). The highest level of visceral fat deposition was seen in the Fat FO fish followed by the Fat VO and Lean FO and VO

298 fish with lowest fat in the CAL fish. Liver lipid content was significantly influenced
299 by both group and diet and there was a significant interaction between the factors
300 (Table 4). Liver lipid content was highest in Fat VO fish (4.8%) and lowest in CAL
301 FO fish (3.2%). In both the Fat VO and CAL VO fish the lipid content was higher
302 than in the same fish fed FO although this was not the case for the Lean FO and VO
303 fish. Flesh protein content was also significantly affected by both diet and group
304 (Table 4). The highest flesh protein was seen in the Lean VO fish and the lowest in
305 the Fat FO fish.

306 All flesh fatty acids were significantly affected by diet with the exception of the
307 minor fatty acid 22:1n-9, and a number of fatty acids were also significantly affected
308 by fish group including 16:0, 18:0, 20:2n-6 and 18:4n-3, but only 20:2n-6 showed an
309 interaction between factors (Table 5). As would be expected, the flesh fatty acids
310 reflected the changes in dietary fatty acids. The main effects observed were a slight
311 reduction in total saturated fatty acids, from 23 to 18%, which was balanced by an
312 increase in monounsaturates from 44 to 50% in fish fed VO. In the n-6 fatty acids,
313 18:2n-6 was increased by ~2-fold as was 20:2n-6, 20:3n-6 and total n-6, in the fish
314 fed VO, while ARA was reduced by 50% (Table 5). In the n-3 PUFA, 18:3n-3 was
315 increased almost 3-fold while all other n-3 fatty acids were reduced in fish fed VO.
316 Flesh EPA and DHA were each reduced by 70% in the Lean fish and by 65% in the
317 CAL fish.

318 After 55 weeks, fish from all treatments were placed on a FO finishing diet and
319 flesh DHA + EPA (%) values were measured at 0, 16 and 24 weeks after switching
320 to this diet (Fig. 1). After 16 weeks on the FO finishing diet, DHA + EPA values

were 25% lower in the CAL and Fat fish previously fed VO but only 11% lower in the Lean VO fish, compared to fish fed FO throughout (Fig. 1). After 24 weeks on the FO finishing diet, DHA + EPA values were still 14% lower in the CAL and Fat fish previously fed VO and 11% lower in the Lean fish fed VO, compared to fish fed FO throughout. For each fish group and diet the flesh content of EPA + DHA was calculated as g /140g flesh at 0, 16 and 24 weeks on the FO finishing diet (Fig. 2). The total EPA + DHA available to consumers, in each experimental group, was compared to the weekly intake values for DHA + EPA recommended by the International Society for the Study of Fatty Acids and Lipids (ISSFAL, 2004). After 55 weeks, a 140g portion of the FO-fed fish would provide 49-53% of the ISSFAL recommended weekly intake while the VO-fed fish would provide 13-17% of this value. After 16 weeks on the finishing diet a 140g portion of FO-fed flesh would provide 63-76% of the recommended weekly intake while the VO-fed fish would provide 46-61% of this value. After 24 weeks on the finishing diet a 140g portion of FO-fed flesh would provide 71-83% of the weekly intake while VO-fed fish would provide 63-78% of the recommended value.

4. Discussion

In the present trial the basal diet contained 25% FM compared to commercial salmon feeds in 2006 that contained 35-40% FM. Plant proteins were included at ~45% of the formulation with the major components being maize and wheat glutens, and soy meal and concentrate. These plant proteins have provided good growth in previous FM replacement studies (Kaushik et al., 2004; Espe et al., 2006). The oil component of the base diet was either 100% northern FO or a blend of VOs

(rapeseed/palm/*Camelina;* (5/3/2 by weight). The oil from *Camelina sativa* is low in saturates (~10%) and has a ratio of 18:3n-3/18:2n-6 of ~2:1. In the total MUFA, (~30%) this oil is unusual in that the major fatty acid is 20:1n-9 (~16%), a fatty acid normally abundant in high latitude FO and highly favoured for energy production by the β-oxidation pathway (Henderson, 1996; Stubhaug et al., 2007). *Camelina* is potentially price competitive with the major oilseeds and can grow in arid conditions not tolerated by other oilseeds and requires minimal application of fertiliser and plant protection products (INFORM, 2007).

Numerous studies have been conducted in salmon, using partial or complete replacement of FO with VO, that have shown no reduction in growth or feed conversion (Bell et al., 2003, 2004; Torstensen et al., 2000, 2004a; Bransden et al., 2003; Karalazos et al., 2007) or in some cases improved growth and feed conversion at low water temperatures (Torstensen et al., 2005; Karalazos et al., 2007). Despite the reduction of FM in the present study, compared to the studies above (FM = 31-74%), growth rates were within expected values for cage-cultured salmon over the whole sea water production with SGR, TGC and FCRs values of ~0.94, 3.54 and 1.17, respectively (Bell et al., 2004; Torstensen et al., 2004a, 2005). In a recent study in salmon, FM and FO were both replaced sequentially to provide four diets containing FM/FO inclusion at 62/25, 12/16, 30/6.5 and 12/7, with the 30/6.5 formulation being close to the 100% VO diet in the present study (Torstensen et al., 2008). In this study, SGR values were in the range 0.42-1.06 and FCRs in the range 0.77-0.91. These are similar to the present study with the SGRs being slightly lower, probably due to lower water temperatures, and the FCRs being slightly better due to

367 the earlier trial being a tank trial where feed delivery and consumption can be more
368 closely monitored compared to cage culture. However, the VO diet used in the
369 present study had much lower levels of DHA and EPA (1.5 and 1.2% of total fatty
370 acids) compared to the diets used by Torstensen et al. (2008) that had only 70% FO
371 replacement, and percentages of DHA and EPA of 3.0 and 2.5, respectively. This
372 50% reduction in dietary n-3 LC-PUFA resulted in no apparent reduction in growth
373 compared to the salmon produced by Torstensen et al. (2008).
374 The current study is the first to consider growth parameters and flesh fatty acids in
375 market-size Atlantic salmon from different genetic backgrounds and different
376 potentials for lipid accumulation and metabolism. Significant differences in fish final
377 weight were attributable to fish group and there was also an interaction between
378 group and diet. The largest fish were the Fat and Lean fish fed FO and this might be
379 due in part to these fish being larger than the CAL fish at the start of the trial.
380 However, while the Lean and Fat groups fed VO had lower growth than the same
381 fish fed FO this was not the case for the CAL fish suggesting that the CAL strain
382 may have performed more effectively with the high VO diet. Although no
383 comparable studies on different genetic groups have been reported in Atlantic
384 salmon, some studies have considered strain differences in coho salmon, rainbow
385 trout and other species. In a study where rainbow trout had been selected on carcass
386 adiposity using a non-invasive fat meter, as was also done in the present study, the
387 strains (Fat, control and Lean) were all different from each other, based on muscle
388 lipid content analysed by Soxhlet, in the order Fat > control > Lean (Quillet et al.,
389 2005). No details of diet were provided except that it was commercial diet containing

22% lipid, which would likely contain predominantly FO. In the present trial, differentiation was achieved by a single round of selection. By contrast to the trout strains, the salmon groups showed differences in % VSI and HSI related to both fish group and diet x group interaction (Quillet et al., 2005).

Another study compared growth and fatty acid compositions in two strains of Atlantic salmon parr (landlocked or anadromous) fed 5 different diets varying in relative ratios of olive, sunflower and linseed oils, for 8 weeks (Rollin et al., 2003). They found that growth rate was higher in the landlocked strain but there was no effect due to the different diets, and the impact of the different diets had a more pronounced effect on carcass fatty acid compositions than that of the different strains (Rollin et al., 2003). In the present study, virtually all flesh fatty acids were significantly affected by diet, which is expected, and was seen in similar studies with salmon fed high levels of VO (Torstensen et al., 2000, 2005; Bell et al., 2003, 2004; Tocher et al., 2003). The saturated fatty acids 16:0, 18:0 and total saturates were significantly affected by both fish group and diet with the CAL fish having higher flesh saturates than the Lean and Fat groups, with lowest levels in the Lean fish. Generally this was balanced by the Lean fish having higher n-3 LC-PUFA levels and this would be expected if the Lean fish were lower in total lipid, compared to the other groups, as these fish should contain more polar lipid and less neutral lipid in their flesh stores. Flesh with less neutral and more polar lipid would tend to accumulate more LC-PUFA and less saturates than the opposite situation seen, for example in the Fat FO fish (Sargent et al., 2002). The above effect was not seen in the VO-fed fish as the lipid deposition pattern was altered in the fish fed VO

413 compared to FO. The absence of increased LC-PUFA in the lean VO fish may also
414 have been influenced by the higher 18:2n-6 content in these fish. It is known that
415 18:2n-6 can compete with LC-PUFA for the *sn*-2 position on phospholipids thereby
416 preventing any increase in LC-PUFA in the phospholiopid fraction. Another
417 potential impact on flesh fatty acids could be the relative activities of the biosynthetic
418 pathways that generate LC-PUFA from their C_{18} precursors and this has been
419 investigated previously to include both diet and strain effects (Tocher et al., 2001,
420 2003; Rollin et al., 2003). A number of other studies have observed differences in
421 metabolism, deposition and retention of LC-PUFA in different salmon strains and
422 there is evidence that this is influenced both by genetic as well as environmental
423 effects (Pickova et al., 1999; Peng et al., 2003). While no direct measure of fatty acid
424 metabolism was conducted in the present study, microarray analysis of the liver
425 transcriptome was analysed to assess effects on global gene expression and this will
426 be presented separately.
427 Many studies have now been conducted on FO replacement in salmon and the
428 influence of VO in reducing the flesh levels of LC-PUFA is a well-known effect of
429 such replacement (Torstensen et al., 2000, 2004a,b, 2005, 2008; Rosenlund et al.,
430 2001; Bell et al., 2002, 2003, 2004; Grisdale-Helland et al., 2002; Bransden et al.,
431 2003). Salmon is a species with oily flesh that can deliver significant levels of n-3
432 LC-PUFA to consumers and it is important to maintain a positive image for the
433 species in this respect as the health benefits of increased dietary n-3 LC-PUFA intake
434 in humans are well documented (Ruxton et al., 2005; Hibbeln and Davis, 2009; Park
435 et al., 2009). One way to successfully restore n-3 LC-PUFA is to dilute or wash out

436 the VO-derived fatty acids using a FO finishing diet (Bell et al., 2004; 2005;
437 Torstensen et al., 2004a, 2005) but this has not previously involved different fish
438 genetic groups, that have differences in flesh lipid deposition that may affect the time
439 and efficacy of the wash out process.
440 At 55 weeks sampling there were no differences in % DHA + EPA between the
441 three groups previously fed VO. However, after 16 weeks of the wash-out process
442 the Lean fish fed VO had significantly higher levels of DHA + EPA compared to the
443 Fat and CAL fish previously fed VO. This suggests that the Lean strain were able to
444 accumulate n-3 LC-PUFA more rapidly than the other two strains, although after 24
445 weeks this difference was abolished. However, this study suggests that LC-PUFA
446 uptake and deposition may be under genetic control and could be exploited to
447 maintain or restore LC-PUFA, following a period of dietary restriction, by selecting
448 for this trait.
449 There is currently interest in increasing fish intake, especially oily fish, as a way
450 of increasing n-3 LC-PUFA levels in populations with traditionally low fish
451 consumption rates. The current advice from ISSFAL is to consume at least 500mg of
452 DHA + EPA/day or 3.5g/week to provide good cardiovascular health in adult
453 humans (ISSFAL, 2004). Consuming a 140g portion of salmon from the present trial,
454 following the 16 week finishing diet period, would provide between 1.62 – 2.67g
455 DHA + EPA (CAL VO and Fat FO, respectively) and, after 24 weeks on the
456 finishing diet, these values increased to 2.21-2.90g/140g portion (Fat VO and CAL
457 FO, respectively). The current guideline of the UK Food Standards Agency
458 (www.food.gov.uk) suggests we should consume two to four 140g portions of oily

fish/week. Consuming two x 140g portions of salmon produced in the current trial, after a 16 week finishing diet period, would easily exceed the ISSFAL recommended weekly intake for DHA + EPA of 3.5g.

5. Conclusions

This study has demonstrated that Atlantic salmon can be cultured using diets with reduced FM and complete replacement of FO, over the seawater production phase, without any detriment to growth and feed conversion. Fish fed the VO diets had n-3 LC-PUFA levels reduced by ~65% compared to fish fed FO although this could be effectively restored by a 16 week FO finishing diet phase. A novel aspect of this study was the use of fish from different genetic backgrounds to investigate differences in growth and fatty acid deposition/retention. Differences were seen between groups due to diet as well as interactions between fish group and diet. The differences observed suggested that genetic background had an influence on lipid deposition and metabolism and that selective breeding programmes could be used to select salmon families that retained more n-3 LC-PUFA in their flesh, particularly when fed diets low in these fatty acids.

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References

Ackman, R.G., 1980. Fish lipids. In: Connell, J. J.(Ed.). Advances in Fish Science and Technology: Fishing News Books, Farnham, pp. 83-103.

AOAC, 2000. Official methods of analysis. Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.

Bell, J.G., 2008. Alternative lipid sources for fish feed and farmed fish quality. In: (Ø. Lie Ed.) Improving farmed fish quality and safety. ISBN 978-1-84569-019-9, Woodhead Publishing Ltd., Cambridge, England. pp. 275-299.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R.P., Sargent, J.R., 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar)* affects muscle fatty acid composition and hepatic fatty acid metabolism. J. Nutr.132, 222-230.

Bell, J.G., Henderson, R.J., Tocher, D.R., Sargent, J.R., 2004. Replacement of dietary fish oil with increasing levels of linseed oil: Modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar)* using a fish oil finishing diet. Lipids 39, 223-232.

505 Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001.
506 Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*)
507 affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr, 131,
508 1535-1543.
509 Bell J.G., Tocher D.R., 2009. Farmed Fish: The impact of diet on fatty acid compositions
510 (chapter 9), In: Rossell B. (Ed.) Oils and Fats Handbook volume 4; Fish Oils, ISBN
511 978-1905224-63-0, Leatherhead Food International, Leatherhead, pp. 171-184.
512 Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R., Crampton, V.O., 2003. Altered fatty
513 acid compositions in Atlantic salmon (*Salmo salar)* fed diets containing linseed and
514 rapeseed oils can be partially restored by a subsequent fish oil finishing diet. J. Nutr.
515 133, 2793-2801.
516 Bourneuf, E., Herault, F., Chicault, C., Carre, W., Assaf, S., Monnier, A., Mottier, S.,
517 Lagarrigue, S., Douaire, M., Mosser, J., Doit, C., 2006. Microarray analysis of
518 differential gene expression in the liver of lean and fat chickens. Gene 372, 162-170.
519 Bransden, M.P., Carter, C.G., Nichols, P.D., 2003. Replacement of fish oil with
520 sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth
521 performance, tissue fatty acid composition and disease resistance. Comp. Biochem.
522 Physiol. 135B, 611-625.
523 Christie, W.W., 2003. Lipid Analysis, 3rd Edition; The Oily Press, Bridgewater, UK. pp.
524 205-224.
525 de Francesco, M., Parisi, G., Medale, F., Kaushik, S.J., Poli, B.M., 2004. Effect of long
526 term feeding with a plant protein mixture based diet on growth and body/fillet quality
527 traits of large rainbow trout (*Oncorhynchus mykiss*). Aquaculture 263, 413–429.

528 Espe, M., Lemme, A., Petri, A., El-Mowafi, A., 2006. Can Atlantic salmon grow on diets
529 devoid of fish meal? Aquaculture 255, 255-262.
530 FAO 2009. The state of world fisheries and aquaculture, 2008. FAO Fisheries and
531 Aquaculture Department. Food and Agriculture Organization of the United Nations,
532 Rome.
533 FAO 2008. Fisheries and Aquaculture Information and Statistics Service. Aquaculture
534 production 1950-2006. FISHSTAT Plus- Universal software for fishery statistical
535 time series [online or CD-ROM]. Food and Agriculture Organization of the United
536 Nations. Available at: http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp
537 Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and
538 purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.
539 Ghioni, C., Bell, J.G., Sargent, J.R., 1996. Polyunsaturated fatty acids in neutral lipids and
540 phospholipids of some freshwater insects. Comp. Biochem. Physiol. 114B, 161-170.
541 Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S.J., Sandberg, M.G.,
542 Standal, H., Roesjoe, C., 2002. Influence of high contents of dietary soybean oil on
543 growth, feed utilization, tissue fatty acid composition, heart histology and standard
544 oxygen consumption of Atlantic salmon (*Salmo salar)* raised at two temperatures.
545 Aquaculture 207, 311-329.
546 Henderson, R.J., 1996. Fatty acid metabolism in freshwater fish with particular reference
547 to polyunsaturated fatty acids. Arch. Anim. Nutr. 49, 5-22.
548 Hibbeln, J.R., Davis, J.M., 2009. Considerations regarding neuropsychiatric nutritional
549 requirements for intakes of omega-3 highly unsaturated fatty acids. Prostaglandins,
550 Leukotrienes Essent. Fatty Acids 81, 179-186.

INFORM (International News on Fats, oils and Related Materials) 2007. Another potential oil source for biodiesel. 18, 386.

Karalazos, V., Bendiksen, E.Å., Dick, J.R., Bell, J.G., 2007. Effects of dietary protein and fat level and rapeseed oil on growth and tissue fatty acid composition and metabolism in Atlantic salmon (*Salmo salar* L.) reared at low water temperatures. Aquacult. Nutr. 13, 256-265.

Kause, A., Quinton, C.D., Ruohonen, K., Koskela, J., 2009. Genetic potential for the regulation of variability in body lipid and protein content of European whitefish (*Coregonus lavaretus).* Br. J. Nutr. 101, 1444-1451.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 133, 257–274.

Kaushik, S.J., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230, 391-404.

Mennicken, L., Ponsuksili, S., Tholen, E., Khang, N.T.K., Steier, K., Petersen, J., Schellander, K., Wimmers, K., 2005. Divergent selection for omega 3 :omega 6 polyunsaturated fatty acid ratio in quail eggs. Arch. Anim. Breeding 48, 527-534.

Neira, R., Lhorente, J.P., Araneda, C., Diaz, N., Bustos, E., Alert, A., 2004. Studies on carcass quality traits in two populations of Coho salmon (*Oncoryhchus kisutch)*: phenotypic and genetic parameters. Aquaculture 241, 117-131.

National Research Council (NRC), 1993. Nutrient requirements of fish. National Academy Press,Washington D.C.

Park, Y., Lim, J., Lee, J., Kim, S-G., 2009. Erythrocyte fatty acid profiles can predict acute non-fatal myocardial infarction. Br. J. Nutr. 102, 1355-1361.

Peng, J., Larondelle, Y., Pham, D., Ackman, R.G., Rollin, X., 2003. Polyunsaturated fatty acid profiles of whole body phospholipids and triacylglycerols in anadromous and landlocked Atlantic salmon (*Salmo salar* L.) fry. Comp. Biochem. Physiol. 134B, 335-348.

Pickova, J., Kiessling, A., Petterson, A., Dutta, P.C., 1999. Fatty acid and carotenoid composition of eggs from two nonanadromous Atlantic salmon stocks of cultured and wild origin. Fish Physiol. Biochem. 21, 147-156.

Pike, I.H., Barlow, S.M., 2003. Impact of fish farming on fish stocks. In: International Aquafeed- Directory and Buyers Guide 2003, pp. 24-29. Turret West, Barthham Press, Luton, England.

Quillet, E., Le Guillou, S., Aubin, J., Fauconneau, B., 2005. Two-way selection for muscle lipid content in pan-size rainbow trout (*Oncorhynchus mykiss).* Aquaculture 245, 49-61.

Richard, N., Kaushik, S., Larroquet, L., Panserat, S., Corraze, G. 2006. Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss).* Br. J. Nutr. 96, 299-309.

595 Rollin, X., Peng, J., Pham, D., Ackman, R.G., Larondelle, Y., 2003. The effects of dietary
596 lipid and strain difference on polyunsaturated fatty acid composition and conversion
597 in anadromous and landlocked salmon (*Salmo salar* L.) parr. Comp. Biochem.
598 Physiol. 134B, 349-366.
599 Rosenlund, G., Obach, A., Sandberg, M.G., Standal, H., Tveit, K., 2001. Effect of
600 alternative lipid sources on long-term growth performance and quality of Atlantic
601 salmon (*Salmo salar* L.). Aquacult. Res. 32, 323-328.
602 Ruxton, C.H.S., Calder, P.C., Reed, S.C., Simpson, M.J.A., 2005. The impact of long-
603 chain polyunsaturated fatty acids on human health. Nutr. Res. Rev. 18, 113-129.
604 Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The Lipids. In: (Halver, J.E., and Hardy,
605 R.W. Eds.) Fish Nutrition, Third Edition. pp. 181-257. Elsevier Science (USA).
606 Stubhaug, I., Lie, Ø., Torstensen, B.E., 2007. Fatty acid productive value and β-oxidation
607 capacity in Atlantic salmon tissues (*Salmo salar* L.) fed on different lipid sources
608 along the whole growth period. Aquacult. Nutr. 13, 145-155.
609 Tacon, A.G.J., Hasan, M.R., Subasinghe, R.P., 2006. Use of fishery resources as feed
610 inputs to aquaculture development: trends and policy implications. FAO Fisheries
611 Circular No. 1018, FAO Rome 99pp.
612 Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in
613 industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285,
614 146-158.
615 Tacon, A.G.J., Nates, S.F., 2007. Meeting feed supply challenges. In: Arthur, R.,
616 Nierentz, J. (Eds.). Global Trade Conference on Aquaculture. Qingdao, China, 29-31
617 May 2007. FAO Fisheries Proceedings, vol. 9. FAO, Rome, 271pp.

618 Tobin, D., Kause, A., Mantysaari, E.A., Martin, S.A.M., Houlihan, D.F., Dobly, A.,
619 Kiessling, A., Rungruangsak-Torrissen, K., Ritola, O., Ruohonen, K., 2006. Fat or
620 lean? The quantitative genetic basis for selection strategies of muscle and body
621 composition traits in breeding schemes of rainbow trout (*Oncorhynchus mykiss).*
622 Aquaculture 261, 510-521.
623 Tocher, D.R., Bell, J.G., Dick, J.R., Crampton, V.O., 2003. Effects of dietary vegetable
624 oil on Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid
625 compositions. Lipids 38, 723-732.
626 Tocher, D.R., Bell, J.G., MacGlaughlin, P., McGhee, F., Dick, J.R., 2001. Hepatocyte
627 fatty acid desaturation and polyunsaturated fatty acid composition of liver in
628 salmonids: effects of dietary vegetable oil. Comp. Biochem. Physiol. 130B, 257-270.
629 Torstensen, B.E., Bell, J.G., Sargent, J.R., Rosenlund, G., Henderson, R.J., Graff, I.E.,
630 Lie, Ø., Tocher, D.R. 2005. Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid
631 composition and sensory quality by replacing fish oil with a vegetable oil blend. J.
632 Agric. Food Chem. 53, 10166-10178.
633 Torstensen, B.E., Espe, M., Sanden, M., Stubaug, I., Waagbø, R., Hemre, G.-I.,
634 Fontanillas, R., Nordgarden, U., Hevrøy, E.M., Olsvik, P., Berntssen, M.H.G., 2008.
635 Novel production of Atlantic salmon (*Salmo salar)* protein based on combined
636 replacement of fish meal and fish oil with plant meal and vegetable oil blends.
637 Aquaculture 285, 193-200.
638
639

Torstensen, B.E., Frøyland, L., Lie, Ø., 2004a. Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil - Effects on Atlantic salmon (*Salmo salar*) tissue and lipoprotein composition and lipogenic enzyme activities. Aquacult. Nutr. 10, 175-192.

Torstensen, B.E., Frøyland, L., Ørnsrud, R., Lie, Ø., 2004b. Tailoring of a cardioprotective fillet fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. Food Chem. 87, 567-580.

Torstensen, B.E., Lie, O., Froyland, L., 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.) – Effects of capelin oil, palm oil and oleic acid – enriched sunflower oil as dietary lipid sources. Lipids, 35, 653-664.

Young, G., Conquer, J., 2005. Omega-3 fatty acids and neuropsychiatric disorders. Reprod. Nutr. Dev. 45, 1-28.

663 **Figure legends**

664 **Fig. 1.** Flesh total lipid 22:6n-3 + 20:5n-3 concentrations (weight % of total fatty acids)
665 in the three salmon strains and two dietary treatments at final sampling and after 16 and
666 24 weeks on a 100% FO finishing diet. Error bars represent mean ± SD. Columns
667 assigned a different letter, within each time point, are significantly different ($P < 0.05$).
668
669 **Fig. 2.** The concentration of flesh 22:6n-3 + 20:5n-3 (g/140g) in the three salmon strains
670 and two dietary treatments at final sample and after 16 and 24 weeks on a FO finishing
671 diet. The dotted line represents the weekly intake values for DHA + EPA for humans
672 recommended by the International Society for the Study of Fatty Acids and Lipids
673 (ISSFAL).
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